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(FILE 'HOME' ENTERED AT 10:15:37 ON 21 JUL 2003)

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3022	FILE SCISEARCH
547	FILE TOXCENTER
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404	FILE VETU
663	FILE WPIDS
663	FILE WPINDEX

L1 QUE XYLANASE

FILE 'CAPLUS, BIOSIS, SCISEARCH, BIOTECHDS, PASCAL, CABA, LIFESCI, BIOTECHNO, AGRICOLA, EMBASE, MEDLINE, ESBIOBASE, FSTA' ENTERED AT 10:17:33 ON 21 JUL 2003

L2 3183 S L1 AND (MUTANT OR VARIANT OR MODIF?)
L3 51 S L2 AND DISULFIDE
L4 20 DUP REM L3 (31 DUPLICATES REMOVED)
L5 2 S L2 AND 162H
L6 2 DUP REM L5 (0 DUPLICATES REMOVED)
L7 461 S L2 AND TRICHODERMA
L8 7 S L7 AND (162H OR POSITION 162 OR 162)
L9 4 DUP REM L8 (3 DUPLICATES REMOVED)

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L9 ANSWER 1 OF 4 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-01501 BIOTECHDS
TITLE: Bleaching of chemical pulp involves, exposing chemical pulp to acidic bleaching stage to produce partially bleached pulp and treating with thermophilic, alkalophilic **xylanase** in alkaline extraction stage at preset condition; pulp bleaching using recombinant enzyme
AUTHOR: TOLAN J; POPOVICI C; FOODY P J
PATENT ASSIGNEE: IOGEN BIO PROD CORP
PATENT INFO: WO 2002052100 4 Jul 2002
APPLICATION INFO: WO 2001-CA1837 19 Dec 2001
PRIORITY INFO: US 2000-258163 22 Dec 2000; US 2000-258163 22 Dec 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-599582 [64]

AB DERWENT ABSTRACT:

NOVELTY - A chemical pulp is bleached by exposing pulp to an acidic bleaching stage to produce a partially bleached pulp and treating with a thermophilic, alkalophilic **xylanase** in an alkaline extraction stage with a final pH of 8-14.

BIOTECHNOLOGY - Preferred Enzyme: The thermophilic, alkalophilic **xylanase** comprises a genetically **modified** **xylanase**, comprising a family 11 **xylanase** from **Trichoderma**. The **xylanase** is a genetically **modified Trichoderma** reesei, selected from Trx HML 75A, 105H, 125A, 129E, 132R, 135R, 144R, 157D, 161R, **162H**, 165H; TrxHML 75A, 105H, 125A, 135R, 144R, 157D, 161R, **162H**, 165H; TrxHML 75A, 105H, 125A, 129E; and TrxHML 75A, 105H, 125A, 129E, 135R, 144R, 157D, 161R, **162H**, 165H (each sequence having 190 amino acids given in the specification), where HML denotes the mutations 10H, 27M and 29L. The **xylanase** comprises BioBrite **xylanase** or a wild type **xylanase**. Preferred Method: The alkaline extraction is performed at 60-120degreesC at a final pH of 9-11.5 for 30-120 minutes. The alkaline extraction is performed using oxygen and/or hydrogen peroxide. 0.1-10 kg of oxygen and hydrogen peroxide is present per ton of pulp. The partially bleached pulp is treated with a second **xylanase** at pH 8-14. The second **xylanase** is identical to the first **xylanase**. The pulp is treated with the first **xylanase** after alkaline oxygen delignification stage. The enzymatic treatment is performed in condition different from the alkaline extraction stage. Alternately, the chemical pulp is exposed to a chemical bleaching stage to produce a partially bleached pulp. The partially bleached pulp is incubated with an extraction filtrate containing the **xylanase** and subsequently washed with water to produce a papricycle washed **xylanase** treated pulp. The papricycle pulp is treated with the **xylanase** at a final pH of 8-14. Then the extraction filtrate is removed from the extract.

USE - For bleaching pulp using **xylanase**.

ADVANTAGE - The method enables to ensure proper mixing of the enzyme with pulp, to control and monitor process conditions such as pH, temperature, enzyme dosage and incubation time. The method does not necessarily require significant changes to existing pulp bleaching equipment, such as purchasing and implementing costly vessels for performing **xylanase** treatment. By carrying out **xylanase** treatment in an alkaline extraction stage, little or no acid is required to adjust the pH of the pulp prior to **xylanase** addition. The reduction or elimination of acid reduces corrosion of mill equipment and the costs associated with a pulp bleaching process. The addition of **xylanase** after an acidic bleaching stage, or before and after a bleaching stage increases the overall effect of enzyme treatment. The pulp bleaching method also reduce the amount of chemicals required to bleach pulp and also reduce the amount of chlorinated effluent waste produced by a pulp bleaching process.

EXAMPLE - Unbleached hardwood kraft pulp was incubated at 60 degrees C, at initial pH 9.4 for 60 minutes to simulate the conditions of an enzyme treatment stage. The pulp was washed with water. 15 g of sample of pulp was subjected to chlorine dioxide bleaching stage. Chlorine dioxide was added to the pulp and the system was maintained in a heat-sealable plastic bag. The pulp mixture was cooled to 4 degrees C to minimize evaporation. The kappa factor was recommended to be about 0.17 to avoid formation of furans and dioxins. The pulp was adjusted to a 10% consistency with tap water and the initial pH was adjusted to 9.4 with sodium hydroxide. The pulp was heated to 60degreesC and a genetically **modified Trichoderma** reesei **xylanase** (having Trx HML 75A, 105H, 125A, 129E, 132R, 135R, 144R, 157D, 161R, **162H** , 165H at amount of 2.0 units/g of pulp with the enzyme stock at 33 units/ml was added to the pulp. Pulp was treated in a similar manner but with a thermophilic, alkalophilic, **xylanase** in the alkaline extraction stage and exhibited a kappa number of 4.8. (61 pages)

L9 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 2001:886467 CAPLUS
DOCUMENT NUMBER: 136:32692
TITLE: Genetic engineering of the **Trichoderma** reesei **xylanase** II for improving thermostability and alkalophilicity for pulp bleaching and other industrial applications
INVENTOR(S): Sung, Wing L.
PATENT ASSIGNEE(S): National Research Council of Canada, Can.
SOURCE: PCT Int. Appl., 109 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001092487	A2	20011206	WO 2001-CA769	20010531
WO 2001092487	A3	20020926		
WO 2001092487	C2	20021205		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
FI 2002002120	A	20021202	FI 2002-2120	20021202
SE 2002003555	A	20030129	SE 2002-3555	20021202

PRIORITY APPLN. INFO.: US 2000-213803P A1 20000531
WO 2001-CA769 W 20010531

AB The present invention pertains to **modified xylanase** enzymes that exhibit increased thermostability and alkalophilicity, when compared with their native counterparts. Several **modified xylanases** exhibiting these properties are disclosed including **xylanases** with at least one **modification** at amino acid position (10, 27, 29, 75, 104, 105, 125, 129, 132, 135, 144, 157, 161, **162**, 165) or a combination thereof. Also included within the present invention is a **modified xylanase** that comprise at least one substituted amino acid residue and that may be characterized as having a max. effective temp. (MET) between about 69.degree. to about 78.degree., wherein the **modified xylanase** is a Family 11 **xylanase** obtained from a **Trichoderma** sp. The present invention also includes a **modified** Family 11

xylanase obtained from a **Trichoderma** sp. characterized as having a max. effective pH (MEP) between about 5.8 to about 7.6. **Modified xylanases** characterized as having a MET between about 69.degree. to about 78.degree. and a MEP between about 5.8 to about 7.6 are also disclosed. The present invention is directed to the use of the **modified xylanase** as defined above in an industrial process. Also included is an industrial process, wherein the industrial process comprises bleaching of pulp, processing of precision devices, or improving digestibility of poultry and swine feed.

L9 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:351671 CAPLUS
DOCUMENT NUMBER: 133:14087
TITLE: Thermostable **xylanase variants** for use in animal feeds
INVENTOR(S): Sung, Wing L.; Tolan, Jeffrey S.
PATENT ASSIGNEE(S): Iogen Corporation, Can.
SOURCE: PCT Int. Appl., 86 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000029587	A1	20000525	WO 1999-CA1093	19991116
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, DE, DK, EE, ES, FI, GB, GE, GH, GM, KE, KG, KZ, LK, LR, LS, MX, NO, PL, PT, RO, RU, SD, SE, SG, SI, SK, US, VN, YU, ZA				
RW: GH, GM, KE, LS, MW, SD, SL, UG, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, IE, IT, LU, NL, PT, SE, NE, SN, TD, TG				
EP 1131447	A1	20010912	EP 1999-972259	19991116
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

PRIORITY APPLN. INFO.: US 1998-108504P P 19981116
WO 1999-CA1093 W 19991116

AB The present invention is directed to thermostable **xylanase** enzymes that are suitable for feed pelleting applications. The novel **xylanase** enzymes comprise at least 40% of their optimal activity from a pH range from about pH 3.5 to about pH 6.0, and from about 40 to about 60.degree., and exhibit at least 30% of their optimal activity after a pre-incubation step for 30 min at 70.degree. in the presence of 40% glycerol. Also disclosed are **modified xylanase** mols. comprising either a basic amino acid at **position 162** (**Trichoderma** reesei **xylanase** (TrX) numbering), or its equiv. position in other **xylanase** mols., at least one disulfide bridge, or a combination thereof. The thermostable **xylanase** mols. of the present invention have a physiol. temp. and pH optima and are useful as animal feeds additives since they can withstand the heat assocd. with feed sterilization and pellet formation, yet they exhibit optimal activity within an animal to aid in breakdown of ingested feed.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 4 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2
ACCESSION NUMBER: 2001:55857 BIOSIS
DOCUMENT NUMBER: PREV200100055857
TITLE: Influence of different grains supplemented with carbohydrases in diets for laying hens.
Original Title: Zur Wirkung verschiedener Getreidearten mit Carbohydrasen-Zusatz im Futter fuer Legehennen..
AUTHOR(S): Jamroz, Dorota (1); Skorupinska, Jolanta; Orda, J.;

Wiliczkiewicz, A.; Kluenter, Anne-Marie
CORPORATE SOURCE: (1) Dept. of Animal Nutrition and Feed Quality, Agricultural University, ul. Chelmonskiego 38 D, 51-639, Wroclaw Poland
SOURCE: Archiv fuer Gefluegelkunde, (Dezember, 2000) Vol. 64, No. 6, pp. 255-263. print.
ISSN: 0003-9098.
DOCUMENT TYPE: Article
LANGUAGE: German
SUMMARY LANGUAGE: English; German
AB The objective of the present experiment was to test whether performance of laying hens and parameters of egg shell quality may be influenced by supplementing wheat-, barley- and rye-based diets with carbohydrases. The experiment was carried out with Hisex Brown laying hens over a period of 268 days. **162** young hens were randomly divided into three groups, each consisted of 9 cages, 3 birds per cage in a 3-floor battery system. Birds were given free access to drinking water. All birds were fed semi ad libitum with the different mixtures. The crude protein level amounted to 15.0%, metabolizable energy to 11.3 MJ/kg, Ca 3.2% and total-P 0.57%. The control group was fed a diet with 60% of wheat (W), birds of group II and III were fed diets containing 60% barley (B) or 50% rye (R), respectively. The diets of groups IV, V, VI were based on the same composition as diets I, II and III but were supplemented with 100 ppm of an enzyme preparation (**Trichoderma** viride) containing beta-glucanase, **xylanase** and cellulase. Laying performance, feed utilization and parameters of egg shell quality were investigated. Laying performance obtained in the experiment reached an average of 92.3 (W), 91.3 (B) and 89.0% (R). The best total egg production was obtained when the hens were fed a diet containing wheat supplemented with the enzyme. Feed intake was not changed in a large scope, the feed utilization calculated on the total mass of produced eggs was slightly better in the groups fed diets supplemented with enzyme. Feed utilization was on average 2.04 kg per 1 kg of egg mass. The parameters of egg shell quality were not **modified** by kind of grains or enzyme supplementation. Strength of egg shell was positively influenced by the enzyme, the number of the cracked eggs was reduced.

L4 ANSWER 19 OF 20 CAPLUS COPYRIGHT 2003 ACS on STNDUPLICATE 8
ACCESSION NUMBER: 1995:224803 CAPLUS
DOCUMENT NUMBER: 122:26699
TITLE: Thermostabilization of the Bacillus circulans **xylanase** by the introduction of **disulfide** bonds
AUTHOR(S): Wakarchuk, Warren W.; Sung, Wing L.; Campbell, Robert L.; Cunningham, Anna; Watson, David C.; Yaguchi, Makoto
CORPORATE SOURCE: Inst. Biological Sciences, Natl. Res. Council Canada, Ottawa, K1A OR6, Can.
SOURCE: Protein Engineering (1994), 7(11), 1379-86
CODEN: PRENE9; ISSN: 0269-2139
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The thermostability of the 20 396 Da Bacillus circulans **xylanase** was increased by the introduction of both intra- and intermol. **disulfide** bridges by site-directed mutagenesis. Based on the 3-D structure of the enzyme, sites were chosen where favorable geometry for a bridge existed; in one case, to obtain favorable geometry addn. mutations around the cysteine sites were designed by computer modeling. The **disulfide** bonds introduced into the **xylanase** were mostly buried and, in the absence of protein denaturants, relatively insensitive to redn. by dithiothreitol. The **mutant** proteins were examd. for residual enzymic activity after various thermal treatments, and were assayed for enzymic activity at elevated temps. to assess their productivity. We have examd. one of these **mutants** by X-ray crystallog. All of the **disulfide** bond designs tested increased the thermostability of the B. circulans **xylanase**, but not all enhanced the activity of the enzyme at elevated temps.

L4 ANSWER 18 OF 20 CAPLUS COPYRIGHT 2003 ACS on STNDUPLICATE 7
ACCESSION NUMBER: 1995:314241 CAPLUS
DOCUMENT NUMBER: 122:100519
TITLE: Stable **mutants** of low molecular mass family G **xylanases**
INVENTOR(S): Campbell, Robert L.; Rose, David R.; Sung, Wing L.; Yaguchi, Makoto; Wakarchuk, Warren W.; Ishikawa, Kazuhiko
PATENT ASSIGNEE(S): National Research Council of Canada, Can.
SOURCE: PCT Int. Appl., 204 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9424270	A2	19941027	WO 1994-CA180	19940407
WO 9424270	A3	19941222		
W: BR, CA, FI, JP, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5405769	A	19950411	US 1993-44621	19930408

PRIORITY APPLN. INFO.: US 1993-44621 19930408

AB The title B. circulans **xylanase** analogs are claimed. The stability of the 20,396 dalton Bacillus circulans **xylanase** was increased by site-directed mutagenesis. Increased stability was conferred by the presence of non-native **disulfide** bridges, and selected N-terminal mutations. The introduction of these non-native **disulfide** bridges was accomplished by the examn. of the three-dimensional structure of the enzyme, and choosing sites where a favorable geometry for a bridge existed. The N-terminal mutations were constructed on the basis of primary sequence comparison with other family G **xylanases**. The **mutant** proteins were examd.: for their ability to retain enzymic activity after heating, as an induction of increased thermostability; for their ability to function at elevated temps. and for their ability to function at a more basic pH. These more stable **variants** are useful as an alternative to chem. bleaching of Kraft pulp in a pre-bleaching step (bio-bleaching). The pre-bleaching step involves higher temp. and pH than that normally used for these enzymes, and accordingly these **variants** can be advantageously used at this step. These stables **xylanases** are also of use in the food processing industry.

L4 ANSWER 13 OF 20 CAPLUS COPYRIGHT 2003 ACS on STNDUPLICATE 5

ACCESSION NUMBER: 1998:463962 CAPLUS
DOCUMENT NUMBER: 129:186086
TITLE: Scan-rate dependence in protein calorimetry: the reversible transitions of Bacillus circulans **xylanase** and a **disulfide**-bridge **mutant**
AUTHOR(S): Davoodi, Jamshid; Wakarchuk, Warren W.; Surewicz, Witold K.; Carey, Paul R.
CORPORATE SOURCE: Department of Biochemistry, University of Ottawa, Ottawa, ON, K1H 8M5, Can.
SOURCE: Protein Science (1998), 7(7), 1538-1544
CODEN: PRCIEI; ISSN: 0961-8368
PUBLISHER: Cambridge University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The stabilities of Bacillus circulans **xylanase** and a **disulfide**-bridge-contg. **mutant** (S100C/N148C) were investigated by differential scanning calorimetry (DSC) and thermal inactivation kinetics. The thermal denaturation of both proteins was found to be irreversible, and the apparent transition temps. showed a considerable dependence upon scanning rate. In the presence of low (nondenaturing) concns. of urea, calorimetric transitions were obsd. for both proteins in the second heating cycle, indicating reversible denaturation occurs under those conditions. However, even for these reversible processes, the DSC curves for the wild-type protein showed a scan-rate dependence that was similar to that in the absence of urea. Calorimetric thermograms for the **disulfide mutant** were significantly less scan-rate dependent in the presence of urea than in the urea-free buffer. The present data show that, just as for irreversible transitions, the apparent transition temp. for the reversible denaturation of proteins can be scan-rate dependent, confirming the prediction of Lepock et al. (Lepock JR, Rithcie KP, Kolios MC, Rodahl AM, Heinz KA, Kruuf J, 1992, Biochem. 31:12706-12712). The kinetic factors responsible for scan-rate dependence may lead to significant distortions and asymmetry of endotherms, esp. at higher scanning rates. This points to the need to check for scan-rate dependence, even in the case of reversible denaturation, before any attempt is made to analyze asym. DSC curves by std. thermodn. procedures. Expts. with the **disulfide** -bridge-contg. **mutant** indicate that the introduction of the **disulfide** bond provides addnl. stabilization of **xylanase** by changing the rate-limiting step on the thermal denaturation pathway.

L4 ANSWER 12 OF 20 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1998:477632 BIOSIS
DOCUMENT NUMBER: PREV199800477632
TITLE: Thermophilic **xylanase** from Thermomyces lanuginosus: High-resolution X-ray structure and modeling studies.
AUTHOR(S): Gruber, Karl; Klintschar, Gerd; Hayn, Marianne; Schlacher, Anton; Steiner, Walter; Kratky, Christoph (1)
CORPORATE SOURCE: (1) Institut Physikalische Chemie, Universitaet Graz, Heinrichstrasse 28, A-8010 Graz Austria
SOURCE: Biochemistry, (Sept. 29, 1998) Vol. 37, No. 39, pp. 13475-13485.
ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The crystal structure of the thermostable **xylanase** from Thermomyces lanuginosus was determined by single-crystal X-ray diffraction. The protein crystallizes in space group P21, a = 40.96(4) ANG, b = 52.57(5) ANG, c = 50.47 (5) ANG, beta = 100.43(5)degree, Z = 2. Diffraction data were collected at room temperature for a resolution range of 25-1.55 ANG, and the structure was solved by molecular replacement with the coordinates of **xylanase** II from Trichoderma reesei as a search model and refined to a crystallographic R-factor of 0.155 for all observed reflections. The enzyme belongs to the family 11 of glycosyl hydrolases (Henrissat, B., and Bairoch, A. (1993) Biochem. J. 293, 781-788). pKa calculations were performed to assess the protonation state of residues relevant for catalysis and enzyme stability, and a heptaxylan was fitted into the active-site groove by homology modeling, using the published crystal structure of a complex between the Bacillus circulans **xylanase** and a xylotetraose. Molecular dynamics indicated the central three sugar rings to be tightly bound, whereas the peripheral ones can assume different orientations and conformations, suggesting that the enzyme might also accept xylan chains which are branched at these positions. The reasons for the thermostability of the T. lanuginosus **xylanase** were analyzed by comparing its crystal structure with known structures of mesophilic family 11 **xylanases**. It appears that the thermostability is due to the presence of an extra **disulfide** bridge, as well as to an increase in the density of charged residues throughout the protein.

L4 ANSWER 7 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 3

ACCESSION NUMBER: 2001:370613 CAPLUS
DOCUMENT NUMBER: 135:192098
TITLE: A combination of weakly stabilizing mutations with a **disulfide** bridge in the .alpha.-helix region of Trichoderma reesei endo-1,4-.beta.-**xylanase** II increases the thermal stability through synergism
AUTHOR(S): Turunen, O.; Etuaho, K.; Fenel, F.; Vehmaanpera, J.; Wu, X.; Rouvinen, J.; Leisola, M.
CORPORATE SOURCE: Laboratory of Bioprocess Engineering, Helsinki University of Technology, 02015, Finland
SOURCE: Journal of Biotechnology (2001), 88(1), 37-46
CODEN: JBITD4; ISSN: 0168-1656
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Thermal stability and other functional properties of Trichoderma reesei endo-1,4-.beta.-**xylanase** II (XYNII; family 11) were studied by designed mutations. Mutations at three positions were introduced to the XYNII **mutant** contg. a **disulfide** bridge (S110C-N154C) in the .alpha.-helix. The **disulfide** bridge increased the half-life of XYNII from less than 1 min to 14 min at 65.degree.C. An addnl. mutation at the C-terminus of the .alpha.-helix (Q162H or Q162Y) increased the half-life to 63 min. Mutations Q162H and Q162Y alone had a stabilizing effect at 55.degree.C but not at 65.degree.C. The mutations N11D and N38E increased the half-life to about 100 min. Due to the stabilizing mutations the pH stability increased in a wide pH range, but at the same time the activity decreased both in acidic and neutral-alk. pH, the pH optimum being at pH region 5-6. There was no essential difference between the specific activities of the **mutants** and the wild-type XYNII.

L4 ANSWER 5 OF 20 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2001-09742 BIOTECHDS
TITLE: Genetically **modified** G/11 **xylanase** used in pulp bleaching, textile fiber **modification** and an animal feed production has increased thermostability and pH stability;
involving vector plasmid pALK143, plasmid pKKtac-mediated gene transfer for expression in Escherichia coli
AUTHOR: Fenel F; Turunen O; Leisola M
PATENT ASSIGNEE: Carbozyme
LOCATION: Helsinki, Finland.
PATENT INFO: WO 2001027252 19 Apr 2001
APPLICATION INFO: WO 2000-FI877 12 Oct 2000
PRIORITY INFO: FI 2000-1586 3 Jul 2000; FI 1999-2186 12 Oct 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-282019 [29]

AB A **modified** endo-1,4-beta-D-**xylanase** (I, EC-3.2.1.8) of family G/11 has increased thermostability or pH-stability and the wild-type enzyme is **modified** by: binding the N-terminal region to the enzyme body by **disulfide** bridges; and/or binding the C-terminal region to the enzyme body salt; or binding the alpha-helix to the enzyme body by salt bridges; or making single amino acid mutations in the enzyme. (I) is used in pulp bleaching, textile fiber **modification**, and **modification** of biomass to improve it digestion in foodstuff. (I) is specifically the Trichoderma reesei **xylanase** II. (I) is prepared by site-directed mutagenesis using the polymerase chain reaction. (I) has amino acid substitutions which impart increased thermostability and pH-stability. T. reesei **xylanase** II was produced in Escherichia coli strains XL1-Blue or Rv308 using the vector plasmid pKKtac or plasmid pALK143. (26pp)

=> d l6 ibib ab 1-2

L6 ANSWER 1 OF 2 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-01501 BIOTECHDS
TITLE: Bleaching of chemical pulp involves, exposing chemical pulp to acidic bleaching stage to produce partially bleached pulp and treating with thermophilic, alkalophilic **xylanase** in alkaline extraction stage at preset condition; pulp bleaching using recombinant enzyme
AUTHOR: TOLAN J; POPOVICI C; FOODY P J
PATENT ASSIGNEE: IOGEN BIO PROD CORP
PATENT INFO: WO 2002052100 4 Jul 2002
APPLICATION INFO: WO 2001-CA1837 19 Dec 2001
PRIORITY INFO: US 2000-258163 22 Dec 2000; US 2000-258163 22 Dec 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-599582 [64]
AB DERWENT ABSTRACT:

NOVELTY - A chemical pulp is bleached by exposing pulp to an acidic bleaching stage to produce a partially bleached pulp and treating with a thermophilic, alkalophilic **xylanase** in an alkaline extraction stage with a final pH of 8-14.

BIOTECHNOLOGY - Preferred Enzyme: The thermophilic, alkalophilic **xylanase** comprises a genetically **modified** **xylanase**, comprising a family 11 **xylanase** from Trichoderma. The **xylanase** is a genetically **modified** Trichoderma reesei, selected from Trx HML 75A, 105H, 125A, 129E, 132R, 135R, 144R, 157D, 161R, **162H**, 165H; TrxHML 75A, 105H, 125A, 135R, 144R, 157D, 161R, **162H**, 165H; TrxHML 75A, 105H, 125A, 129E; and TrxHML 75A, 105H, 125A, 129E, 135R, 144R, 157D, 161R, **162H**, 165H (each sequence having 190 amino acids given in the specification), where HML denotes the mutations 10H, 27M and 29L. The **xylanase** comprises BioBrite **xylanase** or a wild type **xylanase**. Preferred Method: The alkaline extraction is performed at 60-120degreesC at a final pH of 9-11.5 for 30-120 minutes. The alkaline extraction is performed using oxygen and/or hydrogen peroxide. 0.1-10 kg of oxygen and hydrogen peroxide is present per ton of pulp. The partially bleached pulp is treated with a second **xylanase** at pH 8-14. The second **xylanase** is identical to the first **xylanase**. The pulp is treated with the first **xylanase** after alkaline oxygen delignification stage. The enzymatic treatment is performed in condition different from the alkaline extraction stage. Alternately, the chemical pulp is exposed to a chemical bleaching stage to produce a partially bleached pulp. The partially bleached pulp is incubated with an extraction filtrate containing the **xylanase** and subsequently washed with water to produce a papricycle washed **xylanase** treated pulp. The papricycle pulp is treated with the **xylanase** at a final pH of 8-14. Then the extraction filtrate is removed from the extract.

USE - For bleaching pulp using **xylanase**.

ADVANTAGE - The method enables to ensure proper mixing of the enzyme with pulp, to control and monitor process conditions such as pH, temperature, enzyme dosage and incubation time. The method does not necessarily require significant changes to existing pulp bleaching equipment, such as purchasing and implementing costly vessels for performing **xylanase** treatment. By carrying out **xylanase** treatment in an alkaline extraction stage, little or no acid is required to adjust the pH of the pulp prior to **xylanase** addition. The reduction or elimination of acid reduces corrosion of mill equipment and the costs associated with a pulp bleaching process. The addition of **xylanase** after an acidic bleaching stage, or before and after a bleaching stage increases the overall effect of enzyme treatment. The pulp bleaching method also reduce the amount of chemicals required to

bleach pulp and also reduce the amount of chlorinated effluent waste produced by a pulp bleaching process.

EXAMPLE - Unbleached hardwood kraft pulp was incubated at 60 degrees C, at initial pH 9.4 for 60 minutes to simulate the conditions of an enzyme treatment stage. The pulp was washed with water. 15 g of sample of pulp was subjected to chlorine dioxide bleaching stage. Chlorine dioxide was added to the pulp and the system was maintained in a heat-sealable plastic bag. The pulp mixture was cooled to 4 degrees C to minimize evaporation. The kappa factor was recommended to be about 0.17 to avoid formation of furans and dioxins. The pulp was adjusted to a 10% consistency with tap water and the initial pH was adjusted to 9.4 with sodium hydroxide. The pulp was heated to 60degreesC and a genetically **modified** Trichoderma reesei **xylanase** (having Trx HML 75A, 105H, 125A, 129E, 132R, 135R, 144R, 157D, 161R, **162H**, 165H at amount of 2.0 units/g of pulp with the enzyme stock at 33 units/ml was added to the pulp. Pulp was treated in a similar manner but with a thermophilic, alkalophilic, **xylanase** in the alkaline extraction stage and exhibited a kappa number of 4.8. (61 pages)

L6 ANSWER 2 OF 2 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-09767 BIOTECHDS
TITLE: **Modified xylanase** exhibiting increased thermostability and alkalophilicity useful for industrial processing e.g. for pulp manufacturing; vector-mediated gene transfer and expression in host cell for recombinant protein production and feedstuff manufacture
AUTHOR: SUNG W L
PATENT ASSIGNEE: NAT RES COUNCIL CANADA
PATENT INFO: WO 2001092487 6 Dec 2001
APPLICATION INFO: WO 2000-CA769 31 May 2000
PRIORITY INFO: US 2000-213803 31 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-171435 [22]
AB DERWENT ABSTRACT:

NOVELTY - A **modified xylanase** (I) exhibiting increased thermostability and alkalophilicity, comprises at least one substituted amino acid residue at position 75, 104, 105, 125, 129, 132, 135, 144, 157, 161, 162 or 165, where the position is determined from sequence alignment of (I) with a 190 residue Trichoderma reesei **xylanase** II amino acid sequence (S1), fully defined in the specification, is new.

DETAILED DESCRIPTION - A **modified xylanase** (I) exhibiting increased thermostability and alkalophilicity, comprises at least one substituted amino acid residue at position 75, 104, 105, 125, 129, 132, 135, 144, 157, 161, 162 or 165, where the position is determined from sequence alignment of (I) with a 190 residue Trichoderma reesei **xylanase** II amino acid sequence (S1), fully defined in the specification, is new. (I) is characterized as having a maximum effective temperature (MET) of 69-78 degrees C, and maximum effective pH (MEP) of 5.8-7.6, where (I) is a family 11 **xylanase** obtained from a Trichoderma sp..

BIOTECHNOLOGY - Preferred **Variant**: (I) exhibits improved thermophilicity and/or alkalophilicity, in comparison to a corresponding native **xylanase**. The substituted amino acid is at position 75 and is selected from non-polar and a polar amino acid e.g. Ala, Cys, Gly and Thr. (I) is derived from a family 11 **xylanase** e.g. T.reesei **xylanase**. (I) further comprises a His at position 10, Met at position 27 and Leu at position 29 (HML). (I) comprises at least one substituted polar amino acid residue at position 105, e.g. His, Lys and Arg. (I) further comprises a second substituted non-polar or polar amino acid residue at position 75. The polar amino acids at positions 161, 162 and 165 are selected from Arg, Lys and His, and the polar amino acid at

position 157 is selected from Asp and Glu. (I) further comprises at least one substituted non-polar amino acid, Ala, at position 125, and a second substituted acidic amino acid, Glu, at position 129. (I) further comprises a third substituted non-polar amino acid residue (Ala, Cys, Gly and Thr), at position 75, and a fourth substituted polar amino acid residue (His, Lys, and Arg) at position 105. A fifth substituted non-polar amino acid residue, Pro, is present at position 104. Fifth-eleventh substituted polar amino acid residues at positions 132, 135, 144, 157, 161, 162 and 165 are also provided. The MET is 70-75 degrees C, and The pH of MEP is 6.5-7.4. (I) is further characterized as having a maximum effective pH (MEP) is 5.8-7.6. (I) is preferably selected from TrX-161R-**162H**-165H; TrX-HML-75A; TrX-HML-105H; TrX-HML-105R; TrX-HML-105K; TrX-HML-75A-105H; TrX-HML-75A-105R; TrX-HML-75C-105R; TrX-HML-75G-105R; TrX-HML-75T-105R; TrX-HML-125A; TrX-HML-125A-129E; TrX-HML-75G-105R-125A-129E (TrX-HML-GRAE); TrX-HML-75A-105H-125A-129E (TrX-HML-AHAE); TrX-HML-75G-105H-125A-129E (TrX-HML-GHAE); TrX-HML-75A-105R-125A-129E (TrX-HML-ARAE); TrX-HML-75G-104P-105R-125A-129E (TrX-HML-GPRAE); TrX-HML-75G-104P-105H-125A-129E (TrX-HML-GPHAE); TrX-HML-AHAE-RR; TrX-HML-AHAE-RRR; TrX-HML-AHAE-RRR-DRHH; TrX-HML-AHA-RR-DRHH; and TrX-HML-AHAE-RR-DRHH.

USE - (I) is useful in industrial process such as pulp manufacturing (claimed). (I) is also useful for bleaching of pulp, processing of precision devices and improving digestibility of poultry and swine feed.

ADVANTAGE - (I) has improved performance at conditions of high temperature and pH, and exhibits improved thermophilicity and/or alkalophilicity in comparison to a corresponding native **xylanase**

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EXAMPLE - For the construction of TrX(92-190), ten overlapping oligonucleotides XyTv-101, XyTv-102, TrX-103, XyTv-104, XyTv-105, XyTv-106, XyTv-107, TrX-108, XyTv-109, XyTv-110, were designed with codon usage frequency imitating that of Escherichia coli. The salI and BglII cohesive ends of two terminal oligonucleotides enabled the enzymatic ligation of the ten fragments into the linearized plasmid pXYbc. The ten oligonucleotides encoding the TrX(92-190) region of Trichoderma **xylanase** were phosphorylated in a mixture containing 10X standard kinase buffer (0.4 micro-L), 1 mM ATP (4 micro-L), T4 DNA kinase (5 units), and water (3 micro-L). Phosphorylation reactions were carried out for 1 hour at 37 degrees C. The solutions were then combined and heated to 70 degrees C for 10 minutes. After being cooled slowly to room temperature, the combined solutions were added to a mixture of 4 mM ATP (3.5 micro-L), EcoRI-HindIII linearized plasmid pUC119 and T4 DNA ligase (3.5 micro-L), and incubated at 12 degrees C for 20 hours. Aliquots of the ligation mixture were used to transform E. coli HB101 on YT plates containing ampicillin (100 mg/L). For the preparation of a hybridization probe, one of the oligonucleotides, e.g. XyTv-110 (1 micro-L) was phosphorylated with 32P-ATP (3 micro-L) using T4 DNA kinase (1 microL), 10X kinase buffer (1 micro-L), and water (4 micro-L) at 37 degrees C for 1 hour. Transformants were selected randomly for hybridization analysis. Colonies were grown on YT plates with ampicillin overnight, and transferred onto nylon filters. They were then denatured and neutralized. After ultraviolet irradiation at 254 nm for 8 minutes, the filters were washed with 6X saline sodium chloride (SSC)-0.05 % Triton X-100 for 30 minutes. Positively hybridized clones with the intermediate plasmid pBcX-TrX were identified by auto-radiographic analysis.(109 pages)

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IBM Technical Disclosure Bulletins

Term: 12 same disulfide

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L4	l1 same disulfide	32	L4
L3	L2 same (162 or position 162 or 162H)	26	L3
L2	L1 same (mutant or variant or modif$)	413	L2
L1	xylanase	2199	L1

END OF SEARCH HISTORY

1: Protein Expr Purif. 1993 Jun;4(3):200-6. Related Articles, Links

Entrez PubMed

ELSEVIER SCIENCE FULL-TEXT/ARTICLE

Overexpression of the Bacillus subtilis and circulans xylanases in Escherichia coli.

Sung WL, Luk CK, Zahab DM, Wakarchuk W.

Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario.

PubMed Services

An efficient expression system for a low-molecular mass xylanase in Escherichia coli has been developed. A gene encoding the mature Bacillus circulans (Bc) xylanase was designed to imitate the frequency of degenerate codons used in E. coli. Appropriate degenerate codons were used to create multiple unique restriction sites for future mutagenesis studies. The synthetic gene was constructed in two stages, both involving ligation of overlapping oligonucleotides. The synthetic Bc gene was then converted to a Bacillus subtilis (Bs) xylanase gene via a single codon substitution (Thr147Ser). The plasmids containing both synthetic genes were further modified for the direct expression in E. coli. Under the control of the lac promoter, recombinant xylanase has been produced at levels as high as 300 mg/liter in soluble form in the cytoplasm. This efficiency represented a dramatic improvement over all previous attempts involving the expression of the natural genes, with the xylanase being secreted in those cases. Characterization of our gene products indicated that the purified recombinant product was correctly processed and enzymatically active.

PMID: 8518560 [PubMed - indexed for MEDLINE]

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Search Results - Record(s) 1 through 26 of 26 returned.

1. Document ID: US 20030087794 A1

L3: Entry 1 of 26 File: PGPB May 8, 2003

PGPUB-DOCUMENT-NUMBER: 20030087794
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030087794 A1

TITLE: Liquid laundry detergent compositions having enhanced clay removal benefits

PUBLICATION-DATE: May 8, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Price, Kenneth Nathan	Wyoming	OH	US	
Meyer, Axel	Cincinnati	OH	US	

US-CL-CURRENT: 510/499

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

2. Document ID: US 20020165111 A1

L3: Entry 2 of 26 File: PGPB Nov 7, 2002

PGPUB-DOCUMENT-NUMBER: 20020165111
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020165111 A1

TITLE: LAUNDRY DETERGENT COMPOSITIONS COMPRISING ZWITTERIONIC POLYAMINES AND XYLOGLUCANASE

PUBLICATION-DATE: November 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ghosh, Chanchal Kumar	West Chester	OH	US	

US-CL-CURRENT: 510/320; 510/319, 510/392, 510/499

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

3. Document ID: US 20020123445 A1

L3: Entry 3 of 26 File: PGPB Sep 5, 2002

PGPUB-DOCUMENT-NUMBER: 20020123445
PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020123445 A1

TITLE: Stability enhancing formulation components, compositions and laundry methods employing same

PUBLICATION-DATE: September 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Dykstra, Robert Richard	Cleves	OH	US	
Gustwiller, Marc Eric	Cincinnati	OH	US	
Howard, Tonya Ann	South Lebanon	OH	US	

US-CL-CURRENT: 510/302; 510/309, 510/375, 510/499

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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4. Document ID: US 20020010122 A1

L3: Entry 4 of 26 File: PGPB Jan 24, 2002

PGPUB-DOCUMENT-NUMBER: 20020010122
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020010122 A1

TITLE: Liquid laundry detergent compositions having enhanced clay removal benefits

PUBLICATION-DATE: January 24, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Price, Kenneth Nathan	Wyoming	OH	US	
Meyer, Axel	Cincinnati	OH	US	

US-CL-CURRENT: 510/374; 510/499, 510/504

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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5. Document ID: US 20020004474 A1

L3: Entry 5 of 26 File: PGPB Jan 10, 2002

PGPUB-DOCUMENT-NUMBER: 20020004474
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020004474 A1

TITLE: Laundry detergent compositons comprising hydrophobically modified polyamines and nonionic surfactants

PUBLICATION-DATE: January 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Price, Kenneth Nathan	Cincinnati	OH	US	

US-CL-CURRENT: 510/375; 510/376, 510/499

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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6. Document ID: US 6579839 B2

L3: Entry 6 of 26 File: USPT Jun 17, 2003

US-PAT-NO: 6579839
DOCUMENT-IDENTIFIER: US 6579839 B2

TITLE: Liquid laundry detergent compositions having enhanced clay removal benefits

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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7. Document ID: US 6573234 B1

L3: Entry 7 of 26 File: USPT Jun 3, 2003

US-PAT-NO: 6573234
DOCUMENT-IDENTIFIER: US 6573234 B1

TITLE: Liquid detergent compositions comprising polymeric suds enhancers

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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8. Document ID: US 6566114 B1

L3: Entry 8 of 26 File: USPT May 20, 2003

US-PAT-NO: 6566114
DOCUMENT-IDENTIFIER: US 6566114 B1

TITLE: Mannanases

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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9. Document ID: US 6528476 B1

L3: Entry 9 of 26 File: USPT Mar 4, 2003

US-PAT-NO: 6528476
DOCUMENT-IDENTIFIER: US 6528476 B1

TITLE: Liquid detergent compositions comprising block polymeric suds enhancers

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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10. Document ID: US 6525012 B2

L3: Entry 10 of 26 File: USPT Feb 25, 2003

US-PAT-NO: 6525012
DOCUMENT-IDENTIFIER: US 6525012 B2

TITLE: Liquid laundry detergent compositions having enhanced clay removal benefits

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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11. Document ID: US 6503876 B1

L3: Entry 11 of 26 File: USPT Jan 7, 2003

US-PAT-NO: 6503876
DOCUMENT-IDENTIFIER: US 6503876 B1

TITLE: Stable non-aqueous liquid laundry detergents comprising low density particles

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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12. Document ID: US 6486112 B1

L3: Entry 12 of 26 File: USPT Nov 26, 2002

US-PAT-NO: 6486112
DOCUMENT-IDENTIFIER: US 6486112 B1

TITLE: Laundry detergent compositions comprising a saccharide gum degrading enzyme

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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13. Document ID: US 6479451 B2

L3: Entry 13 of 26 File: USPT Nov 12, 2002

US-PAT-NO: 6479451
DOCUMENT-IDENTIFIER: US 6479451 B2

TITLE: Laundry detergent compositions comprising hydrophobically modified polyamines and nonionic surfactants

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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14. Document ID: US 6472359 B1

L3: Entry 14 of 26 File: USPT Oct 29, 2002

US-PAT-NO: 6472359
DOCUMENT-IDENTIFIER: US 6472359 B1

TITLE: Laundry detergent compositions comprising zwitterionic polyamines and xyloglucanase

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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15. Document ID: US 6440911 B1

L3: Entry 15 of 26 File: USPT Aug 27, 2002

US-PAT-NO: 6440911
DOCUMENT-IDENTIFIER: US 6440911 B1

TITLE: Enzymatic cleaning compositions

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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16. Document ID: US 6420331 B1

L3: Entry 16 of 26 File: USPT Jul 16, 2002

US-PAT-NO: 6420331
DOCUMENT-IDENTIFIER: US 6420331 B1

TITLE: Detergent compositions comprising a mannanase and a bleach system

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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17. Document ID: US 6384008 B1

L3: Entry 17 of 26 File: USPT May 7, 2002

US-PAT-NO: 6384008
DOCUMENT-IDENTIFIER: US 6384008 B1

TITLE: Non-aqueous liquid detergent compositions containing ethoxylated quaternized amine clay compounds

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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18. Document ID: US 6376445 B1

L3: Entry 18 of 26 File: USPT Apr 23, 2002

US-PAT-NO: 6376445
DOCUMENT-IDENTIFIER: US 6376445 B1

TITLE: Detergent compositions comprising a mannanase and a protease

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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19. Document ID: US 6228983 B1

L3: Entry 19 of 26 File: USPT May 8, 2001

US-PAT-NO: 6228983
DOCUMENT-IDENTIFIER: US 6228983 B1
**** See image for Certificate of Correction ****

TITLE: Human respiratory syncytial virus peptides with antifusogenic and antiviral

activities

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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20. Document ID: US 6121226 A

L3: Entry 20 of 26 File: USPT Sep 19, 2000

US-PAT-NO: 6121226
DOCUMENT-IDENTIFIER: US 6121226 A
**** See image for Certificate of Correction ****

TITLE: Compositions comprising cotton soil release polymers and protease enzymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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21. Document ID: US 6093794 A

L3: Entry 21 of 26 File: USPT Jul 25, 2000

US-PAT-NO: 6093794
DOCUMENT-IDENTIFIER: US 6093794 A

TITLE: Isolated peptides derived from the Epstein-Barr virus containing fusion inhibitory domains

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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22. Document ID: US 6060299 A

L3: Entry 22 of 26 File: USPT May 9, 2000

US-PAT-NO: 6060299
DOCUMENT-IDENTIFIER: US 6060299 A

TITLE: Enzyme exhibiting mannase activity, cleaning compositions, and methods of use

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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23. Document ID: US 6060065 A

L3: Entry 23 of 26 File: USPT May 9, 2000

US-PAT-NO: 6060065
DOCUMENT-IDENTIFIER: US 6060065 A

TITLE: Compositions for inhibition of membrane fusion-associated events, including influenza virus transmission

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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24. Document ID: US 5395765 A

L3: Entry 24 of 26 File: USPT Mar 7, 1995

US-PAT-NO: 5395765
DOCUMENT-IDENTIFIER: US 5395765 A

TITLE: Thermostable xylanase from a strain of Rhodothermus marinus

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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25. Document ID: SE 200203555 A WO 200192487 A2 AU 200167171 A

L3: Entry 25 of 26 File: DWPI Jan 29, 2003

DERWENT-ACC-NO: 2002-171435
DERWENT-WEEK: 200327

TITLE: Modified xylanase exhibiting increased thermostability and alkalophilicity useful for industrial processing e.g. for pulp manufacturing

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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26. Document ID: WO 200029587 A1 AU 200011451 A EP 1131447 A1

L3: Entry 26 of 26 File: DWPI May 25, 2000

DERWENT-ACC-NO: 2000-387799
DERWENT-WEEK: 200033

TITLE: Thermostable xylanases useful for preparing animal feeds especially poultry or swine feed, exhibits optimal activity under physiological conditions

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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L3	L2 same (162 or position 162 or 162H)	26	L3
L2	L1 same (mutant or variant or modif$)	413	L2
L1	xylanase	2199	L1

END OF SEARCH HISTORY

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L5: Entry 16 of 16 File: DWPI Oct 27, 1994

DERWENT-ACC-NO: 1994-341852
DERWENT-WEEK: 199610

TITLE: Modified family G xylanase with increased stability - for bio:bleaching of paper pulp at increased temperature and pH

Basic Abstract Text (1):
A modified family G xylanase is new. The xylanase has the structure of the Bacillus circulans enzyme and has increased stability caused by introduction of at least one ion-native disulphide bridge, the introduction of at least one N-terminal mutation, or combinations of these. The disulphide bridge is an intramolecular bridge between a Cys which has been introduced on the last strand of B-sheet III, and a Cys which has been introduced on the alpha-helix, or on either side, adjacent to the alpha-helix, or the disulphide bridge is an intermolecular bridge between two xylanase molecules, where the cys has been introduced in each molecule on the external region. The N-terminal mutation is selected from at least one mutation of the group consisting of introducing Tyr or Phe at position 8, Pro at position 22 and the introduction of an N-C-terminal disulphide band. These N-terminal mutations can be used with other N-terminal mutations introduced at positions 1-25 of the N-terminal region.

L5: Entry 15 of 16 File: USPT Apr 11, 1995

DOCUMENT-IDENTIFIER: US 5405769 A
TITLE: Construction of thermostable mutants of a low molecular mass xylanase

Abstract Text (1):
The thermostability of the 20,396 dalton Bacillus circulans xylanase was increased by site-directed mutagenesis. The thermostability was conferred by the presence of non-native disulfide bridges, and selected N-terminal mutations. The introduction of these non-native disulfide bridges was accomplished by the examination of the three-dimensional structure of the enzyme, and choosing sites where a favorable geometry for a bridge existed. The N-terminal mutations were constructed on the basis of primary sequence comparison with other family G xylanases. The mutant proteins were examined for their ability to retain enzymatic activity after heating as an indication of increased thermostability. These thermotolerant variants are useful as an alternative to chemical bleaching of Kraft pulp in a pre-bleaching step (bio-bleaching). The pre-bleaching involves temperatures higher than that normally used for these enzymes and accordingly these thermotolerant variants can be advantageously used at this step. Thermotolerant xylanases are also of use in the food processing industry.

Brief Summary Text (2):
The present invention is directed to a modified xylanase, which shows an improved thermostability when compared to the naturally occurring xylanase. Specifically the present invention is directed to a modified xylanase, wherein said xylanase has increased thermostability and wherein said xylanase is modified through either the introduction of a non-native disulfide bridge, introduction of an N-terminal mutation, or both.

Brief Summary Text (12):
According to the present invention there is provided a modified xylanase, wherein said xylanase has increased thermostability and wherein said xylanase is modified through either the introduction of a non-native disulfide bridge, introduction of an N-terminal mutation, or both. No mutations of any type of xylanase have led to improved thermostability as described in the present invention.

Brief Summary Text (13):
In one embodiment of the present invention, the modified xylanase has been modified by the introduction of an intra-molecular disulfide bridge between a cysteine amino acid, which has been introduced on the last strand of sheet III, and a cysteine amino acid, which has been introduced on the alpha helix.

Brief Summary Text (14):
In a further embodiment of the present invention, the modified xylanase has been modified by the introduction of an inter-molecular disulfide bridge between two xylanase molecules, wherein a cysteine amino acid has been introduced in each of said two molecules.

Drawing Description Text (5):
FIG. 4 shows a stereodiagram of the superimposed structures of the wild-type and disulfide-containing mutant (TS1) of the B. circulans xylanase in the vicinity of the mutation (residues 100 and 148). The wild-type enzyme is drawn in thick lines and the mutant is drawn in thin lines.

Drawing Description Text (11):

FIG. 10 shows the electrophoretic mobility of the disulfide bridge containing mutants of B. circulans xylanase. Lane 1 shows the molecular weight standard, lane 2 is the BCX wild-type, reduced, lane 3 is as in lane 2 but non-reduced, lane 4 is the TS1 mutant, reduced, lane 5 is the TS1 mutant, non-reduced, lane 6 is the TS2 mutant, reduced and lane 7 is a TS2 mutant, non-reduced.

Drawing Description Text (24):
FIG. 23 shows the average main-chain B-factors for the disulfide-containing mutant (TS1) of the xylanase from B. circulans.

Detailed Description Text (37):
A portion of the three-dimensional crystal structure of a mutant of the B. circulans xylanase containing an intramolecular disulfide bond is shown in FIG. 4. In this mutant serine 100 and asparagine 148 have been mutated to cysteine. The disulfide which is formed links the last strand of sheet III to the alpha-helix. The structure of this mutant is identical to the wild-type enzyme except for the side chains of residues 100 and 148 and some very minor shifts of nearby atoms. The same region of the superimposed structures of the B. circulans and T. harzianum xylanases is shown in FIG. 5. Further details of the analyses will be provide in the examples, however, one can see that although there are some sequence differences between the B. circulans and T. harzianum xylanases near the site of the manufactured disulfide and some small position differences at other nearby residues, the structure of the T. harzianum xylanase is virtually identical to the structure of the B. circulans xylanase at the positions of the mutations. The sequence alignment (FIG. 1) shows that the sequence homology of the various xylanases in the vicinity of residues 98 to 100 and 148 to 152 is very good. One would therefore expect that the structures of other related xylanases would be similar enough that they could be modified with the introduction of an intramolecular disulfide bond linking sheet III to the alpha-helix and that this disulfide bond would stabilize them in a similar manner as taught in the present invention. For some xylanases with the lowest sequence homology to the B. circulans xylanase it may be necessary to make other mutations to increase the homology in the vicinity of the introduced intramolecular disulfide bond. In other words it may be necessary to make another xylanase more like the B. circulans xylanase before the disulfide bond could be formed successfully.

Detailed Description Text (41):
In addition to the intra-molecular SS bond mutant, a second type of disulfide mutant was constructed by joining two protein molecules with an inter-molecular SS bond. For the production of this type of disulfide bond, a cysteine is introduced in each of the two xylanase protein molecules such that the cysteine is on the exterior of the molecule, thus the two molecules can be joined by an inter-molecular disulphide bond. Potential sites of interest include amino acid 15 to 31 of the beta-sheet I; amino acid 43 to 61 of the beta-sheet I; amino acid 87 to 104 of the loop and beta-sheet III; amino acid 133 to 163 of the alpha-helix and surrounding loops; and amino acids 177 to 185 of the beta-sheet I.

CLAIMS:

1. A modified family G xylanase essentially having the structure of the B. circulans enzyme or mutated to essentially have this structure, wherein said xylanase has increased thermostability and wherein said xylanase is modified at least through either the introduction of a disulfide bridge, introduction of a N-terminal mutation, or both;

wherein the disulfide bridge is an intra-molecular bridge between two cysteine amino acids selected from the group consisting of 100 and 148, and 98 and 152, based on the amino acid numbering from B. circulans, or the disulphide bridge is an intermolecular bridge between two xylanase molecules, wherein a cysteine amino acid has been introduced in each of said two molecules at amino acid position 179, based on the amino acid numbering from B. circulans xylanase; and

wherein the N-terminal mutation introduces a tyrosine or phenylalanine amino acid at amino acid position 3, 4 or 8 of the N-terminal region, or a combination thereof, based on the amino acid numbering from B. circulans xylanase.

8. The modified xylanase of claim 2 wherein said xylanase is modified by the introduction of an intra-molecular disulfide bridge.

9. The modified xylanase of claim 1 wherein said xylanase is modified by the introduction of an inter-molecular disulfide bridge.

11. The modified xylanase of claim 1 wherein said xylanase is modified by the introduction of a intra-molecular disulfide bridge and an inter-molecular disulfide bridge.

12. The modified xylanase of claim 1 wherein said xylanase is modified through the introduction of an intra-molecular disulfide bridge; an inter-molecular disulfide bridge and an N-terminal mutation.

13. The modified xylanase of claim 1 wherein said xylanase is modified through the introduction of an intra-molecular disulfide bridge and an N-terminal mutation.

14. The modified xylanase of claim 1 wherein said xylanase is modified through the introduction of an inter-molecular disulfide bridge and an N-terminal mutation.

15. A modified family G xylanase essentially having the structure of the B. circulans enzyme or mutated to essentially have this structure, wherein said xylanase has increased thermostability and wherein said xylanase is modified at least through either the introduction of a disulfide bridge, introduction of a N-terminal mutation, or both; and wherein said modified xylanase is produced from clones selected from the group consisting of TS1, TS2, TS3a, TS3, TS4a, TS4, TS4M, TS4D, TS5a, and TS6a.

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1: Sung WL, Luk CK, Chan B, Wakarchuk W, Yaguchi M, Campbell R, Willick G, Ishikawa K, Zahab DM. Related Articles, Links

Expression of Trichoderma reesei and Trichoderma viride xylanases in Escherichia coli.
Biochem Cell Biol. 1995 May-Jun;73(5-6):253-9.
PMID: 8829371 [PubMed - indexed for MEDLINE]

2: Wakarchuk WW, Sung WL, Campbell RL, Cunningham A, Watson DC, Yaguchi M. Related Articles, Links

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Protein Eng. 1994 Nov;7(11):1379-86.
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3: Wakarchuk WW, Campbell RL, Sung WL, Davoodi J, Yaguchi M. Related Articles, Links

Mutational and crystallographic analyses of the active site residues of the Bacillus circulans xylanase.
Protein Sci. 1994 Mar;3(3):467-75.
PMID: 8019418 [PubMed - indexed for MEDLINE]

4: Sung WL, Luk CK, Zahab DM, Wakarchuk W. Related Articles, Links

Overexpression of the Bacillus subtilis and circulans xylanases in Escherichia coli.
Protein Expr Purif. 1993 Jun;4(3):200-6.
PMID: 8518560 [PubMed - indexed for MEDLINE]

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Expression of Trichoderma reesei and Trichoderma viride xylanases in Escherichia coli.

Sung WL, Luk CK, Chan B, Wakarchuk W, Yaguchi M, Campbell R, Willick G, Ishikawa K, Zahab DM.

Institute for Biological Sciences, National Research Council of Canada, Ottawa, ON, Canada.

Synthetic genes encoding the 190 amino acid Trichoderma reesei xylanase II (TrX) and the closely related Trichoderma viride xylanases have been synthesized in a two-step procedure. Initially, a partial gene encoding amino acids 92-190 was constructed in fusion with the N-terminal half of the Bacillus circulans xylanase (BcX). The remaining BcX gene sequence was replaced during the assembly of the coding sequence for amino acids 1-91. Expression of the synthetic genes in Escherichia coli yielded recombinant xylanases with specific activity generally identical with the natural TrX. However, the recombinant TrX showed thermostability and temperature optimum lower than those of the natural TrX, thus indicating that the posttranslational modifications of the latter in its fungal host are essential to its greater stability. A mutation N19K further decreased the thermostability of the recombinant TrX.

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Thermostabilization of the Bacillus circulans xylanase by the introduction of disulfide bonds.

Wakarchuk WW, Sung WL, Campbell RL, Cunningham A, Watson DC, Yaguchi M.

Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario.

The thermostability of the 20 396 Da Bacillus circulans xylanase was increased by the introduction of both intra- and intermolecular disulfide bridges by site-directed mutagenesis. Based on the 3-D structure of the enzyme, sites were chosen where favourable geometry for a bridge existed; in one case, to obtain favourable geometry additional mutations around the cysteine sites were designed by computer modelling. The disulfide bonds introduced into the xylanase were mostly buried and, in the absence of protein denaturants, relatively insensitive to reduction by dithiothreitol. The mutant proteins were examined for residual enzymatic activity after various thermal treatments, and were assayed for enzymatic activity at elevated temperatures to assess their productivity. We have examined one of these mutants by X-ray crystallography. All of the disulfide bond designs tested increased the thermostability of the B. circulans xylanase, but not all enhanced the activity of the enzyme at elevated temperatures.

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Mutational and crystallographic analyses of the active site residues of the Bacillus circulans xylanase.

Wakarchuk WW, Campbell RL, Sung WL, Davoodi J, Yaguchi M.

Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario.

Using site-directed mutagenesis we have investigated the catalytic residues in a xylanase from Bacillus circulans. Analysis of the mutants E78D and E172D indicated that mutations in these conserved residues do not grossly alter the structure of the enzyme and that these residues participate in the catalytic mechanism. We have now determined the crystal structure of an enzyme-substrate complex to 108 A resolution using a catalytically incompetent mutant (E172C). In addition to the catalytic residues, Glu 78 and Glu 172, we have identified 2 tyrosine residues, Tyr 69 and Tyr 80, which likely function in substrate binding, and an arginine residue, Arg 112, which plays an important role in the active site of this enzyme. On the basis of our work we would propose that Glu 78 is the nucleophile and that Glu 172 is the acid-base catalyst in the reaction.

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